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REVIEW ARTICLE

Macronutrient effect on biomass of Microalgae in biofuel production: A review

Mee Kin Chai[1]*, Yeong Hwang Tan[1], Ling Shing Wong[2]

1 College of Engineering, University Tenaga Nasional, Jalan Ikram-Uniten, Kajang, 43000, Selangor, Malaysia. Tel.: +603-89287275
2 Faculty of Health and Life Science, INTI International University, Persiaran Perdana BBN, Putra Nilai, Nilai, 71800, Negeri Sembilan, Malaysia

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***Corresponding author**.
Mee Kin Chai

College of Engineering, University Tenaga Nasional, Jalan Ikram-Uniten, Kajang, 43000, Selangor, Malaysia.
Tel.: +603-89287275
mkchai@uniten.edu.my

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Abstract

Objectives: This review is focused on the effect of macronutrients (nitrogen, carbon and phosphorus) on biomass production of microalgae especially concerned with biofuel. **Methodology**: The keyword search included "microalgae cultivation", "nitrogen sources", "phosphorus sources", "organic carbon", "biodiesel", "biofuel", "carbon dioxide", "inorganic carbon", "macronutrient deprivation", "macronutrient limitation", "lipid" and "organic waste" to search the published journals in ScienceDirect, Scopus, Springer, and Google Scholar. The search was performed from December 2019 until Mac 2020 to collect all the journals and books that are published between 2006 and 2020. The effect of each macronutrient (nitrogen, carbon and phosphorus) on microalgal growth of the control and the samples were compared using biomass productivity, concentration and biochemical content in each published article. **Findings**: Review shows that nitrogen has more pernicious effect than other macronutrients on most microalgal growth and lipid production. The concentrations and types of macronutrients have remarkable effects on the growth of microalgae; hence these criteria must be chosen scrupulously to achieve the desired biomass and metabolite production. In order to improve the biomass and biochemical productivity in concomitant with the cost reduction, replacement of cheap organic waste, genetic engineering of microalgae and two-stage hybrid system have been suggested to simultaneously maximize the biomass and biochemical production. The future research should focus on other biochemical contents such as carbohydrates, proteins and pigment to achieve the biorefinery context which can increase the profit. Besides, economic factor such as factorial design should be included in the future research to obtain the best combined factors with the maximum profit and minimal cost.

Keywords: Microalgae; biomass; macronutrient; biofuel

1 Introduction

Microalgae-derived biofuel has several superior advantages over edible plant oil derived biofuel (1). High lipid productivity and fast biomass generation without contend arable land for food production render microalgae become ideal biofuel sources. The vast coastlines including creeks, mangroves and seashore waterlog areas can be used as microalgal arable land. Some microalgae do not necessarily require freshwater to grow and would not exacerbate the global freshwater crisis. Oppositely, some microalgae can effectively remove the pollutants from wastewater (2; 3). Moreover, the desired biochemical content of microalgae can be achieved by altering nutrient composition or environmental conditions and the outcome is feasibly detected within several weeks (4). Despite of lipids, other high biomolecules possessed by microalgae can be converted into high value-added products and biofuel resources.

Previous studies have revealed that factors such as types of microalgae, nutrients composition, types of cultivation medium, carbon dioxide concentration, temperature, photoperiod, light intensity, salinity and pH directly affect the biomass and biochemical content of microalgae (5). Among these factors, nutrient is a prime factor that determine the microalgal growth and metabolites composition. Several reviews have briefly discussed the importance of nutrients on microalgal growth (4; 5; 6); but the mechanism of how these nutrients affect the microalgal growth and metabolites is still untapped. Understanding the effect of nutrients on microalgal growth and its mechanism can help to attain the maximum production efficiency whilst precluding any unnecessary dissipation.

The present review is solely focused on the effect of macronutrients (nitrogen, carbon and phosphorus) on biomass production of microalgae followed by the elucidation of the possible mechanism behind these macronutrients. Therefore, other factors such as temperature, photoperiod, light intensity, pH and salinity conditions are not be a part of this review. The aim of this review is to provide a glimpse for the better understanding on how the macronutrients (nitrogen, carbon and phosphorus) affect the microalgal growth for biofuel production.

In this study, the cited bibliographic references were extracted from published journals and books. The keywords include "microalgae cultivation", "nitrogen sources", "phosphorus sources", "organic carbon", "biodiesel", "biofuel", "carbon dioxide", "inorganic carbon", "macronutrient deprivation", "macronutrient limitation", "lipid" and "organic waste" were used to search the published journals in ScienceDirect, Scopus, Springer, and Google Scholar. The relevant literatures were chosen by scrupulously analyzing abstract and using keywords to search through all the content of literatures. Then, the content of literature was scrutinized to extract the significant information related to this proposed review. The search was performed from December 2019 until Mac 2020 to collect all the related journals and books that are published between 2006 and 2020. As most of the research papers cited were carried out in laboratory with "one-factor-at-a-time", the commentaries in this review might be different with those performed at the outdoor or with factorial design. Moreover, some vocabularies such as nutrient deprivation, limitations and low-concentrations and stress were found out to have similar meaning in different journals.

2 Macronutrients Affecting Microalgal Growth and Biochemical Composition

2.1 Nitrogen

Nitrogen is an essential component of proteins, chlorophylls, nucleic acid, enzymes, and other nitrogen-containing compounds that are indispensable in maintaining the microalgal growth. Extensive studies have indicated that microalgal biomass productivity was decreased during nitrogen starvation or limitation due to perturbation of the cell division and photosynthetic activities. By contrast, nitrogen abundance promotes the cell growth and cell division due to high photosynthesis efficiency (7; 8).

Generally, lipid and carbohydrate storage of microalgae are increased rapidly whereas protein content is plummeted during nitrogen starvation or limitation compared to medium supplemented with abundant nitrogen (7; 9; 10; 11). Interestingly, Li et al. (12) observed varied results which Chlorella vulgaris JNU13 cultivated in medium with nitrogen-repletion was capable to accumulate more lipid content at the late phase of cultivation. Similar results were also reported by Jerez (9) and Kim et al. (13) who used Chlorella fusca BEA1005B and Tetraselmis sp. respectively.

This erratic phenomenon could be explained with continuous aeration of CO_2 or air throughout the cultivation which sufficient carbon source is provided for lipid biosynthesis.

When nitrogen is scarce, either in nitrogen starvation or deprivation, photosynthetic apparatus in photosynthetic system II such as chlorophyll and thylakoid membrane are degraded (9; 14). Accordingly, the flow of electrons from the photosystems to the electron transport chain is impaired, and the reactive oxygen species (ROS) are formed. Antioxidant defense is subsequently activated but ROS are aggregated along with prolonged nitrogen starvation therefore create the permanent damage to the cells (15). Interestingly, these exogenous oxidant stresses induce significantly of the lipid accumulation, especially triacylglycerol (TAG) which is suitable used as biodiesel feedstock (15). On the other hand, Safdar et al. (16) revealed that during the extended nitrogen starvation, enzymes of tricarboxylic acid (TCA) cycle are downregulated whereas enzymes of lipid biosynthesis are upregulated. This in turn redirect substrate of citrate from TCA cycle to lipid biosynthesis (Figure 1). The possible reason behind a trigger in the lipid accumulation under nitrogen starvation might be the requirement of substantial energy of adenosine triphosphate (ATP) and nicotinamide adenine dinucleotide phosphate (NADPH) (17). The TAG synthesis could reduce oxidation stress by serving as a receptor for dissipation of the excess electron of ROS.

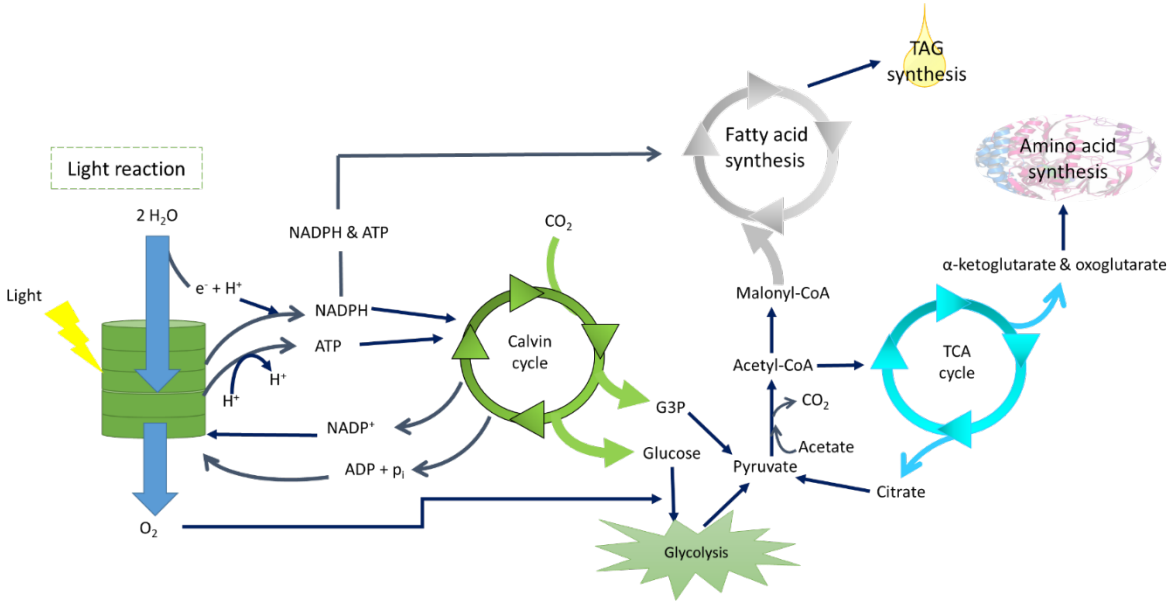

Fig 1. Simplified diagram of microalgae metabolisms (18). G3P: glyceraldehyde-3-phosphate.

Some researchers have also analyzed the effect of different nitrogen sources on microalgal growth and their metabolite composition (Table 1). Most of the microalgae are capable of utilizing nitrate, nitrite, urea and ammonium with different responses on the basis of species (19; 20). Urea is favorable for large-scale microalgae cultivation because of its low cost compared to other sources (21). In several studies, the utilization of nitrate and urea as nitrogen source results in higher biomass and lipid content than that of using ammonium as nitrogen source (20; 21; 22; 23). In contrast, Chlorella variabilis with optimum ammonium concentration was demonstrated to have the better growth than those utilized urea and nitrate. On the downside, the cells growth was reduced beyond optimum ammonium concentration. The inhibitory effect on cell growth by ammonium can be elucidated by two possible reasons. The pH in the medium with ammonium usually is acidic and it is likely attributed to the release of hydrogen ion during ammonium assimilation (24). The acidic environment is unpleasant for most of the microalgal growth. Second, the excessive transport of ammonium to the cells can forbid some enzymes activity and ATP formation in the chloroplast, results in the inhibition of photosynthesis (25).

While very few studies focused on the effect of nitrogen sources on lipid accumulation and composition, the effect on carbohydrate and protein is still untapped. Different nitrogen sources can diversify lipid accumulation and

composition (21; 26; 27; 28) therefore affect biodiesel quality. It is noteworthy to mention that nitrogen sources that superior for microalgal growth are not necessarily promote microalgae to generate fatty acid that suitable for biodiesel. Nannochloropsis salina supplemented with urea was grow faster and had the highest cell density than nitrate and ammonium. However, TAG accumulation was the lowest due to small cell size (29). Moreover, Zhan et al. (24) demonstrated the high lipid accumulation induced by nitrogen sources do not ensure produce high amount of TAG using the same nitrogen sources. Thus, it is imperative to control nitrogen sources and concentrations in order to attain the desirable metabolite amount and composition.

Table 1. Impact of nitrogen source and concentrations on microalgal metabolites change

Microalgae species	Type of medium	Nitrogen sources	Concentration (g/L)	Other specific experiment factor (if available)	Biomass productivity (mg/L/d)	Metabolite (%)			Ref
						Lipid	Protein	Carbs	
Nannochloropsis salina	f/2	$NaNO_3$	18.75	30 °C under 150 μmol photons m^{-2} d^{-1} with 12:12 h (light: dark) photoperiod	0.53 g/L	59.3	-	-	(8)
Nannochloropsis salina	f/2	$NaNO_3$	75	30 °C under 150 μmol photons m^{-2} d^{-1} with 12:12 h (light: dark) photoperiod	0.61 g/L	34.6	-	-	(8)
Chlorella fusca BEA1005B	BG-11	$NaNO_3$	0	1.5 % CO_2 (v/v) in 28-32 °C under 1200 μmol photons m^{-2} s^{-1}	250	27	9	49	(9)
Chlorella fusca BEA1005B	BG-11	$NaNO_3$	75	1.5 % CO_2 (v/v) in 28-32 °C under 1200 μmol photons m^{-2} s^{-1}	820	31	18	29	(9)
Chlorella vulgaris	BG-11	$NaNO_3$	5.8 mM	1 % CO_2 (v/v) in 25 °C under 300 μmol photons m^{-2} s^{-1} with 24h: 0h (light: dark) photoperiod	4750 mg/L	9.5 ρg/cells	0.2 ρg/cells	5.0 ρg/cells	(12)
Chlorella vulgaris	BG-11	$NaNO_3$	17.6 mM	1 % CO_2 (v/v) in 25 °C under 300 μmol photons m^{-2} s^{-1} with 24h: 0h (light: dark) photoperiod	7130 mg/L	12 ρg/cells	2.5 ρg/cells	2.5 ρg/cells	(12)
Tetraselmis sp. KCTC 12236BP	f/2 medium without Na_2SiO_3	$NaNO_3$	0 mM	0.2 vvm air in 20 - 25 °C under 110 - 120 μmol photons m^{-2} s^{-1}	78	19.9	-	-	(13)
Tetraselmis sp. KCTC 12236BP	f/2 medium without Na_2SiO_3	$NaNO_3$	0.88 mM	0.2 vvm air in 20 - 25 °C under 110 - 120 μmol photons m^{-2} s^{-1}	110	21.3	-	-	(13)
Chlorella variabilis	Modified BG-11	$NaNO_3$	1.5	25 ± 2 °C under 3.8 klux with aeration rate of 300 L/h	1300 mg/L	15.2	-	-	(19)
Tetraselmis sp.	Artificial seawater with f/2 nutrient	Yeast extract	8.82 mM	20 - 25 °C under 100 -120 μmol photons m^{-2} s^{-1} with 24 h: 0 h (light: dark) photoperiod	140000	19.6	45.0	19.7	(20)
Tetraselmis sp.	Artificial seawater with f/2 nutrient	$NaNO_3$	8.82 mM	20 - 25 °C under 100 -120 μmol photons m^{-2} s^{-1} with 24 h: 0 h (light: dark) photoperiod	140000	19.6	45.0	19.7	(20)
Monoraphidium sp. SB2	Artificial medium	KNO_3	3.6 mM	pH 6.8, 25 °C under 25 mmol photons m^{-2} d^{-1} with 14:10 h (light: dark) photoperiod and shaken at 120 rpm	93	31.5	-	-	(23)

Continued on next page

Table 1 continued

Microalgae species	Type of medium	Nitrogen sources	Concentration (g/L)	Other specific experiment factor (if available)	Biomass productivity (mg/L/d)	Metabolite (%)			Ref
Chlorella sp. HQ	Modified BG-11	$NaNO_2$	0.015	25 °C under 60 μmol photons m^{-2} s^{-1} with 14 h: 10 h (light: dark) photoperiod	1.87 cells mL^{-1} d^{-1}	44.16	-	-	(24)
Scenedesmus Dimorphus	BG-11	Beef extract	-	25 °C	85.8	30.28	1.94	23.98	(30; 2)
Scenedesmus Dimorphus	BG-11	$NaNO_3$	-	25 °C	144.17	21.40	7.40	23.98	(30)
Ankistrodesmus sp.	ASM-1	$NaNO_3$	0.04	0.01 g/L P and 5.0 g/L NaCl; 22 ± 2 °C under 1.4 mmol photons m^{-2} d^{-1} with 12:12 h (light: dark) photoperiod	18.2	27.6	-	-	(29)
Ankistrodesmus sp.	ASM-1	$NaNO_3$	0.17	0.01 g/L P and 5.0 g/L NaCl; 22 ± 2 °C under 1.4 mmol photons m^{-2} d^{-1} with 12:12 h (light: dark) photoperiod	36.8	18.0	-	-	(29)
Chlamydomonas sp.	ASM-1	$NaNO_3$	0.04	0.01 g/L P and 5.0 g/L NaCl; 22 ± 2 °C under 1.4 mmol photons m^{-2} d^{-1} with 12:12 h (light: dark) photoperiod	45.3	36.5	-	-	(29)
Chlamydomonas sp.	ASM-1	$NaNO_3$	0.17	0.01 g/L P and 5.0 g/L NaCl; 22 ± 2 °C under 1.4 mmol photons m^{-2} d^{-1} with 12:12 h (light: dark) photoperiod	88.0	10.9	-	-	(29)
Scenedesmus vacuolatus	BG-11	$NaNO_3$ and glutamate	10 mM and 1 mM	25 °C under 10 Wm^{-2} with 16 h: 8 h (light: dark) photoperiod	700 mg/L	16.02	130 μg/mL	140 μg/mL	(31)
Chlorella pyrenoidosa	Selenite enrichment medium	NH_4^+	0.28	pH 8.3-8.5, 25 °C under 127 μmol photons m^{-2} s^{-1} with 12 h: 12 h (light: dark) photoperiod	18.5	30.2	46.5	15.5	(32)
Chlorella pyrenoidosa	Modified BG-11	$NaNO_3$	0	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	0.74	52.03	-	-	(33)
Chlorella pyrenoidosa	Modified BG-11	$NaNO_3$	1.5	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	0.97	34.68	-	-	(33)
Synechococcus sp.	Modified BG-11	$NaNO_3$	0	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	0.28	27.41	-	-	(33)
Synechococcus sp.	Modified BG-11	$NaNO_3$	1.5	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	1.81	18.90			(33)
Chlorella sorokiniana	BBM	$NaNO_3$	0.030	air flow of 0.1 vvm, 2% CO_2 and 25 ± 1 °C under 300 μmol photons m^{-2} s^{-1} with 24 h: 24 h (light: dark) photoperiod	450	41.3	41.5	15.1	(34)

2.2 Carbon

Carbon plays a critical role in microalgal growth and biochemical synthesis of microalgae. Carbon sources either in inorganic or organic form can be supplied from microalgae medium. Different sources and concentrations of

carbon have significant effects on the microalgal growth, metabolite content and composition in microalgal cells. In the inorganic form of carbon sources, carbon dioxide (CO_2) is more favorable than bicarbonate salt, attributes to the benefits of greenhouse gas mitigation and low cost (35). The optimal CO_2 concentration for microalgae often falls between the ranges from 2 to 15 % (v/v) and might species-specific (Table 2). Typically, low CO_2 level is insufficient for microalgal growth, whereas high CO_2 level often exerts detrimental effect (35; 36; 37). Chloroplast damage and organelles disorder which in turn render cell lysis were observed in high CO_2 level (38).

Many microalgae have evolved CO_2 concentrating mechanism (CCM) to enhance the efficiency of photosynthetic carbon fixation by raising the CO_2 level around carboxylating enzyme ribulose bisphosphate carboxylase/oxygenase (RuBisCO) which responsible for the first step of carbon dioxide fixation (Figure 2). CCM contains transporter for actively transport bicarbonate ions into the cells and a key enzyme of carbonic anhydrase (CA) that catalyzes interconversion between CO_2 and bicarbonate ion for RuBisCO (39). CCM is induced when the external inorganic carbon is limited. However, carbon source becomes a limiting factor for the grow of microalgae and results in idle of microalgal growth in very low CO_2 level. Besides, the enzyme of RuBisCO from microalgae is known to have very low affinity to CO_2. On the other hand, oxygen gas (O_2), one of the products of photosynthesis also acts as a substrate for RuBisCO. When the ratio of O_2 to CO_2 is high, RuBisCO uses O_2 rather than CO_2 to catalyze the energy wasting photorespiration (40). Hence, the presence of O_2 might compete with low level of CO_2 and avert carbon fixation. Oppositely, high CO_2 level restrains microalgal growth. High CO_2 level usually concomitants with the reduction of pH which ascribes to the dissociation of carbonic acid (H_2CO_3) into carbonate ions (CO_3^{2-}) and hydrogen ion (H^+) (41). Although CCM can get sufficient CO_2 under high CO_2 level condition, previous study implied that the enzymes of CCM are suppressed in acidic pH (42) and this damage is irreversible under prolonged high CO_2 level (43).

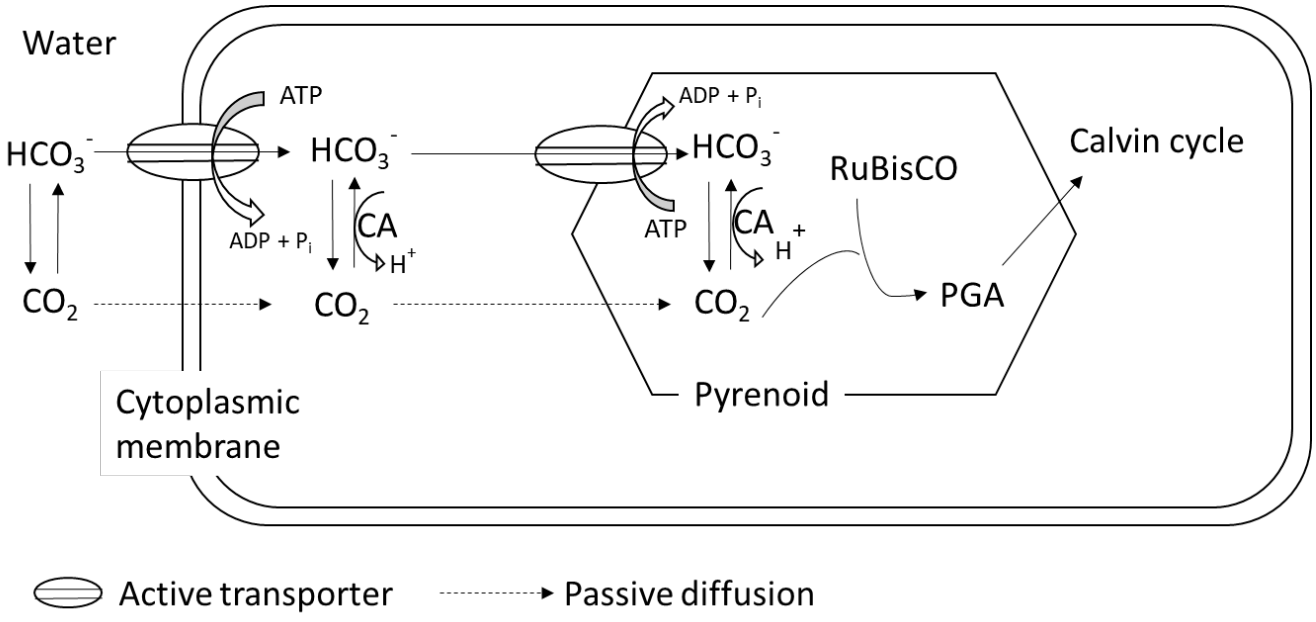

Fig 2. A general diagram for CO2 concentration in microalgae. The diagram is slightly changed from (5) .

The CCM from microalgae is still not clearly explicated especially for those competent to thrive under very high CO_2 level. Three indigenous microalgal isolates viz., Desmodesmus sp., Kirchneriella sp. and Acutodesmus sp isolated through CO_2-tolerance screening can grow in 30% (v/v) of CO_2 level. The biomass concentration, specific growth rate, chlorophyll and carbon dioxide fixation rate were enhanced two to four-fold after a period of sixteen days cultivation (44).

Apart from inorganic carbon, microalgae can harness organic carbon as carbon source. Glucose is a prevalent organic carbon consumed by many microalgae for rapid cell growth and high biochemical accumulation because of its easy assimilation into intermediate product of many metabolic pathway (45; 46; 47). Other organic carbon sources including sucrose (48), glycerol (49), galactose (50), xylose (51), gluconate (52) are also suitable for some microalgae. The utilization of organic carbon is species-dependent, and the effects are summarized in Table 2. In an investigation on the effect of organic sources on marine microalgae of Pavlova lutheri, sucrose was identified as prime organic carbon source for growth, followed by glucose, glycerol and acetate (53). Notwithstanding, some microalgae are devoid of metabolize sucrose. Sharma et al. (54) reported stunt growth was observed in four Chlorella sp. that grew in medium supplemented with sucrose whereas microalgae with optimal growth was ensued from medium supplemented with glucose. The differences on the metabolism of these organic carbon sources in stimulating microalgal growth might be dependent on the availability and activation of suitable hexose transporters such as monosaccharide-H^+ symport to catalyze the transport of sugars across the cell membrane (55; 56). Hexose transporters have been identified in Chlorella sp. but still not imparted in other microalgae species. Moreover, leverage of the organic carbon is also as contingent on the availability of metabolic pathway to transform the organic carbon into usable intermediate product (57).

Similar to inorganic carbon, the concentration of organic carbon sources in culture medium must be carefully modulated. Appropriate amount of organic carbon sources can induce microalgal cell growth and metabolites accumulation whereas excessive amount can decline the growth and metabolites accumulation (53). Danesh et al. (49) cultivated Isochrysis galbana under different concentration of glycerol. The results showed that the cell density and lipid content were reduced at the concentration exceed 25 mM. Besides, Chai et al (58) revealed galactose had no effect on Chlorella sorokiniana growth and lipid accumulation in all tested concentration while xylose had inhibitory effect on C. sorokiniana.

The effects of CO_2 and organic carbon on total amount of microalgal metabolites composition especially lipid have been investigated in literature studies (Table 2). Many researchers (35; 58; 59; 60)proved that appropriate CO_2 level under autotrophic condition stimulated the biosynthesis of lipid content whereas high CO_2 level (> 5 to 10%) stifled lipid accumulation. On the other hand, apparent escalating of lipid content was not observed in microalgae that can thrive in high CO_2 level (44; 61). The intensity of the reduction or enhancement in carbohydrate, protein and lipid composition is species-dependent. Different microalgae responded individually with varied biochemical composition (62; 63; 64). Zhang et al. (60)reported that the protein content in Chlorella pyrenoidosa relatively constant regardless of the change in CO_2 concentration while carbohydrate and lipid content increased with CO_2 concentration up to 3% followed by declined with further increase in CO_2 concentration. Whereas the carbohydrate and lipid content of Scenedesmus bajacalifornicus BBKLP-07 elevated with CO_2 concentration up to 25% while protein content decreased in 20 and 25% of CO_2. Consequently, it is difficult to judge the influence of CO_2 level on these biochemical compositions. Different microalgae species have different cell size, shapes, CCM and growth rate hence their sensitivity to CO_2 concentration is varied. Small or slow growing cells are less sensitive to the declined CO_2 concentration (65). In other word, leverage of CO_2 concentration alone might not an efficient strategy for certain species to stimulate the high microalgal growth with low CO_2 concentration.

The effects of organic carbon sources on the microalgae metabolites on lipid content have been investigated by several studies. However, limited studies focused on the effects of protein and carbohydrate production. Despite of the type of organic carbon source, maximum lipid productivity is also relied on the concentration and presence of light. Moreover, the responses of metabolite accumulation under the conditions thereof are species-dependent (54; 66). Several literature studies advocated the organic carbon that induced maximum microalgal biomass and also exerted maximum lipid production (30; 57; 67; 68; 69). In some cases, organic carbons that induce maximum biomass are not necessary render maximum lipid production. In the scrutiny of organic carbon sources on Monoraphidium minutum, 15 g/L fructose and 15 g/L glucose promoted maximum biomass productivity of M. minutum, however, the maximum lipid production was ensued from 20 g/L fructose and 5 g/L glucose amended medium which induced mediocre biomass productivity (70). The supplementation of 3 g/L glucose has been proved as optimal car-

bon sources for stimulating high cell density of C. pyrenoidosa but contemporaneous with significantly curtailing of lipid and protein content (56). Similar result was also attained by He et al. (71) which glucose promoted the greatest biomass of Scenedesmus sp. LX1 but lowest lipid content. Medium imbued with other sugars such as sucrose, maltose and xylose did not buoy up inferior biomass but high lipid accumulation was occurred in these mediums. This occurrence probably due to the nutrient trauma caused by the organic sugars.

Generally, concentration of organic carbon wields the hormesis effect on microalgal metabolite accumulation. Low concentration of organic carbon has no significant effect on lipid production. On the other hand, lipid production is balked in concentration beyond the optimum (72; 73). Supplementation of 3.0 g/L glucose and galactose notably reduced the lipid content of C. pyrenoidosa by 27.5% and 27.9% (50). Addition of 5.0 g/L glucose remarkably dampened lipid content of Phaeodactylum tricornutum (74). Wan et al. (73) revealed that large amount of glucose still remained in the medium after cultivated with C. sorokiniana. In other word, high concentration of glucose molecules was not transported into microalgal cell and consumed sequentially. Further analysis of gene expression disclosed that the genes coded for lipid and RuBisCO biosynthesis were downregulated in the presence of excessive concentration of glucose. It should be noted that fatty acid composition and amount are varied with the supplementation of different organic carbon sources (70) and different concentrations of carbon source (75).

Table 2. Impact of carbon source and concentrations on microalgal metabolites change

Microalgae species	Type of medium	Carbon sources	Concentration	Other specific experiment factor (if available)	Biomass productivity (mg/L/d)	Metabolite (%)			Ref
						Lipid	Protein	Carbs	
Isochrysis galbana	f/4	CO_2	10%	Open raceway; 10-30 °C under 447 - 1081 μmol photons m^{-2} s^{-1} in photobioreactor	142.42 g/m^2/d	40.7 8	-	45.98	(35)
Nannochloropsis sp.	f/4	CO_2	10%	Open raceway; 10-30 °C under 447 - 1081 μmol photons m^{-2} s^{-1} in photobioreactor	149.92 g/m^2/d	37.54	-	46.88	(35)
Scenedesmus bajacalifornicus	Modified BG-11	CO_2	0.04%	pH 7	27	15.48	23.03	6.88	(37)
Scenedesmus bajacalifornicus	Modified BG-11	CO_2	15%	pH 7	61	20	32.89	20	(37)
Desmodesmus sp.	BG-11	CO_2	10%	25 ± 1°C under 30 μE m^{-2} s^{-1} with 16 h: 8 h (light: dark) photoperiod	97	21.4	-	50.33	(44)
Desmodesmus sp.	BG-11	CO_2	0.03%	25 ± 1°C under 30 μE m^{-2} s^{-1} with 16 h: 8 h (light: dark) photoperiod	25	11.5	-	43.82	(44)
Acutodesmus sp	BG-11	CO_2	20%	25 ± 1°C under 30 μE m^{-2} s^{-1} with 16 h: 8 h (light: dark) photoperiod	98	18	-	49.87	(44)
Acutodesmus sp	BG-11	CO_2	0.03%	25 ± 1°C under 30 μE m^{-2} s^{-1} with 16 h: 8 h (light: dark) photoperiod	32	6.3	-	41.63	(44)
Kirchneriella sp.	BG-11	CO_2	20%	25 ± 1°C under 30 μE m^{-2} s^{-1} with 16 h: 8 h (light: dark) photoperiod	109	14.8	-	50.49	(44)

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Table 2 continued

Kirchneriella sp.	BG-11	CO_2	0.03%	25 ± 1°C under 30 μE m^{-2} s^{-1} with 16 h: 8 h (light: dark) photoperiod	37	9.6	-	47.74	(44)
Chlorella sp. Y8-1	modified Walne	CO_2	10% (2 vvm)	30 °C under 4300 lux with 24 h: 0 h (light: dark) photoperiod	220 mg/L	16.5	-	-	(57)
Scenedesmus quadricauda FACHB-1297	BG-11	CO_2	-	25 ± 1 °C under 60 μmol photons m^{-2} s^{-1}	18.6	20.92	26	-	(72)
Auxenochlorella protothecoides	SAG	CO_2	1 g/L	26 ± 1 °C under 80 μmol photons m^{-2} $s^{-1}$with 18 h: 6 h (light: dark) photoperiod	38	6.84	-	-	(75)
Chlorella vulgaris ESP-31 (wild type)	BG-11	CO_2	25% (0.1 vvm)	Indoor photobioreactor; 40 °C under 300 μE photons m^{-2} s^{-1} with 14 h: 10 h (light: dark) photoperiod	120	3.72	-	-	(76)
Chlorella vulgaris ESP-31 mutant 283	BG-11	CO_2	25% (0.1 vvm)	Indoor photobioreactor; 40 °C under 150 μmol photons m^{-2} s^{-1} with 12 h: 12 h (light: dark) photoperiod	420	17.84	-	29.98	(76)
Chlorella vulgaris CCAP 211/79	BBM with 3x N and vitamin	CO_2	15%	Blue luminescent dye photobioreactor; under 200 μmol photons m^{-2} s^{-1}	90.12	25.6	-	-	(77)
Scenedesmus dimorphus	BB	CO_2	15% (0.26 vvm)	25 ± 1 °C under 150 μmol photons m^{-2} s^{-1} with 12 h: 12 h (light: dark) photoperiod	80	19.6		58.93	(63)
Scenedesmus obliquus	BB	CO_2	14.1%	25 ± 1 °C under 150 μmol photons m^{-2} s^{-1} with 12 h: 12 h (light: dark) photoperiod	45	22.8	-	23.6	(63)
Chlorella vulgaris	BG-11	Glycerol and CO_2	0.5 g/L and 10%	22- 30 °C under 3000 lux with 16 h: 8 h (light: dark) photoperiod	-	24.32	-	-	(54)
Chlorella sp. Y8-1	modified Walne	Sucrose	1 g/L	30 °C without light	170 mg/L	5.9			(57)
Chlorella sp. Y8-1	modified Walne	Sucrose and CO_2	1 g/L and 10% at 2 vvm	30 °C under 4300 lux with 24 h: 0 h (light: dark) photoperiod	450 mg/L	35.5			(57)

Continued on next page

Table 2 continued

Chlorella sp	Modified TAP	Glycerol and CO_2	16 g/L and 0.5%	30 °C under 48 μmol photons m^{-2} $s^{-1}$with 16 h: 8 h (light: dark) photoperiod	1440	43.2	-	-	(66)
Scenedesmus Dimorphus	BG-11	Glucose	1% (w/v)	25 °C without light	180	32.7	1.57	13.88	(30)
Scenedesmus sp. LX1	Modified BG-11	Glucose	10 g/L	pH 7, 25 ± 1 °C without light	156.36	1.28	-	-	(71)
Scenedesmus sp. LX1	Modified BG-11	Sucrose	10 g/L	pH 7, 25 ± 1 °C without light	9.09	26.66	-	-	(71)
Auxenochlorella protothecoides	SAG	Glucose and CO_2	1 g/L	26 ± 1 °C under 80 μmol photons m^{-2} $s^{-1}$with 18 h: 6 h (light: dark) photoperiod	58	19.38	-	-	(75)

2.3 Phosphorus

Phosphorus is an indispensable nutrient for the formation of nucleic acids, phospholipids and energy molecules in microalgal cells. The utilization of phosphorus is species-dependent and the effects are summarized in Table 3. The phosphorus acquisition varies greatly between microalgal species. Compared to nitrogen, phosphorus starvation has little detrimental effect on microalgal growth (29; 78). Similar to nitrogen and carbon, too low concentration of phosphorus unable to support microalgal growth thereby result in reduction of biomass concentration (79). In contrast, when the external phosphorus is abundant, the excess inorganic phosphorus will be deposited as polyphosphate in microalgal cells. In the condition of phosphorus deprivation, microalgae still can anabolize the polyphosphate and continue to grow as long as nitrogen supply is still sufficient. (80; 81; 82; 18; 83; 84). Besides, several types of transporters such as vacuolar transporter chaperone are promoted to facilitate the external phosphate uptake (85; 86; 87).

In addition of external phosphorus uptake, photosynthesis and carbohydrate accumulation during phosphorus starvation have been reported (86; 87). However, when the microalgae initiate stationary phase, chlorophylls are gradually degraded and the genes involved in carbon fixation and glycolysis are upregulated in which acetyl-CoA and NADH are synthesized for storage accumulation of either carbohydrates or lipids or both (84; 87). In this regard, TAG biosynthesis is activated to ingest excess carbon and reduce energy generated from photosynthesis. As a result, TAG is accumulated during phosphorus starvation (85; 86; 87).

Phospholipid is the main component for biosynthesis of microalgal cell membrane (88). In concomitant with metabolisms thereof are carried out, Mühlroth et al. (82) evinced that the genes related to phospholipid degradation were surged in microalgae during phosphorus starvation. In other words, phospholipids are degraded from cell membrane in order to compensate the phosphorus acquisition. Alternatively, synthesis of non-phosphorus lipid including sulfolipids and non-phosphorus glycolipids are diverted to substitute the phospholipid membrane (81; 89). This mechanism might allow the microalgae to grow under phosphorus starvation. On the other hand, phospholipid degradation releases glycerol-3-phosphate, fatty acid and diacylglyceride which could serve as precursors for TAG biosynthesis.

Phosphorus starvation can eventuate to the apparent change in lipid composition. Lipid composition is varied to the microalgal species. Saturated and unsaturated fatty acid in microalgae were gradually increased with the reduction of phosphorus concentration (90). Howbeit, synthesis of saturated and unsaturated fatty acid would be declined if the phosphorus concentration is too low (91; 92). This occurrence is probably due to the low biomass concentration. It worth mentioning that fatty acid content is increased in different extent either in phosphorus starvation with or without nitrogen sources (~0 mg/L). Isochrysis zhangjiangensis has higher amount of fatty acid in phosphorus starvation with nitrogen sources (78) whereas reversed result was attained from Chlorella sp (83). Several studies have investigated the effect of phosphorus on microalgal protein content. Since phosphorus is not a primary element

in the protein, little effects on protein content were observed in phosphorus starvation (83; 86). Protein content did not changed significantly and was slightly lower than the control (93; 94).

Large excessive amount (> ~45 mg/L) of phosphorus results in hormesis effect and hinder microalgal growth (88; 92). Li et al. (88) elucidated that the overabundant storage of polyphosphate granule in Chlorella regularis distorted both cell membrane and cell wall. Meanwhile, Fu et al. (95) revealed that the contorted structure of excess polyphosphate granule was observed concomitant with the mitochondrial and DNA disorder. Consequently, no energy molecules were synthesized to sustain the metabolism which in turn induce the cell death.

Table 3. Impact of phosphorus concentrations on microalgal metabolites change

Microalgae species	Type of medium	P sources	Concentration (mg/L)	Other specific experiment factor (if available)	Biomass productivity (mg/L/d)	Metabolite (%)			Ref
						Lipid	Protein	Carbs	
Chlorella pyrenoidosa	Modified BG-11	K_2HPO_4	0	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	17.22	49.08	-	-	(33)
Chlorella pyrenoidosa	Modified BG-11	K_2HPO_4	40	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	17.22	29.56	-	-	(33)
Synechococcus sp.	Modified BG-11	K_2HPO_4	0	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	16.11	22.81	-	-	(33)
Synechococcus sp.	Modified BG-11	K_2HPO_4	40	pH 8.0, 25 °C under 140 μmol photons m^{-2} s^{-1}	16.11	17.06	-	-	(33)
Rhopalosolen saccatus	ASM	K_2HPO_4 and Na_2HPO_4	0.32	25 °C under 400- 450 μmol photons m^{-2} s^{-1} with aeration of 43 L/min	28.75	19	-	-	(90)
Rhopalosolen saccatus	ASM	K_2HPO_4 and Na_2HPO_4	0.65	25 °C under 400- 450 μmol photons m^{-2} s^{-1} with aeration of 43 L/min	35.83	13	-	-	(90)
Porphyridium purpureum	ASW	K_2HPO_4	0	pH 7.6 under 165 μmol photons m^{-2} s^{-1} with aeration of 1 or 3 L/min (contain 3% CO_2)	425.0	2.32	21.22	36.28	(92)
Porphyridium purpureum	ASW	K_2HPO_4	35	pH 7.6 under 165 μmol photons m^{-2} s^{-1} with aeration of 1 or 3 L/min (contain 3% CO_2)	808.57	5.88	22.26	32.74	(92)
Scenedesmus obliquus	BG-11	K_2HPO_4	14	pH 7.5, 28 ± 2 °C under 180 μE m^{-2} s^{-1}	-	16	27.81	13.72	(93)
Scenedesmus obliquus	BG-11	K_2HPO_4	0.035	pH 7.5, 28 ± 2 °C under 180 μE m^{-2} s^{-1}	-	9	31.18	15.78	(93)
Chlorella sp.	BG-11	K_2HPO_4	32 μM	25 ± 2 °C under 30 μ mol photons m^{-2} s^{-1}	-	23.60	22.50	22.25	(83)
Messastrum gracile	f medium	$Na_2HPO_4 \cdot 2H_2O$	4.54 μM	20 °C under 25 μE m^{-2} s^{-1} with aeration	0.69 g/L	38.1	-	-	(84)
Messastrum gracile	f medium	$Na_2HPO_4 \cdot 2H_2O$	145.2 μM	20 °C under 25 μE m^{-2} s^{-1} with aeration	0.50 g/L	25.4	-	-	(84)
Chaetoceros muelleri	f medium	$NaH_2PO_4 \cdot$	7 μM	22 ± 1 °C under 220 μmol photons m^{-2} s^{-1} without aeration	199.92	4.42	4.29	44.60	(86)

Continued on next page

Table 3 continued

Chaetoceros muelleri	f medium	NaH_2PO_4	144 μM	22 $\pm$ 1 °C under 220 μmol photons m^{-2} s^{-1} without aeration	248.07	3.18	4.22	56.18	(86)

3 Future Recommendations

Microalgae has been identified as potential candidate for biofuel production. To serve the purpose, the lipid content of microalgae have been studied worldwide. Although the microalgae consist of high amount of lipid which is comparable to other oil crops, no commercial production is established until now because of their mass production and harvesting are not cost affordable as compared to fossil diesel (96).

Several hindrances should be tackled to turn microalgae production from lab scale to pilot scale and industrial scale. In order to achieve industrial scale, economical mass production with high amount of biochemical products must be attained. Expensive artificial medium used in lab scale is not feasible for mass production (97). In this scenario, integrating wastewater and flue gas have been employed to cultivate microalgae (4; 98; 99). This integrating approach is not only could offset the capital and operation cost, but also can help the mitigating air and water pollutions. In particular, microalgal cultivation in wastewater is mainly performed at lab scale whereas pilot scale study is still scarce. Besides, several obstacles might be faced in pilot scale study such as presence of invading microorganisms, fluctuating compositions in wastewater, high turbidity and light penetration (100; 101; 102). These issues should be properly tackled in the future study.

Despite of lipids, proteins and carbohydrates can be obtained from microalgae. These metabolites have shown to possess high nutritional value which can be utilized in agricultural application and biogas production (103; 104). Simultaneous production of these metabolites as co-products is another smart tactic to increase the profit. The research relevant to proteins, carbohydrates and pigments should be performed concomitant with the lipid of microalgal study.

Reducing environment pollution is the main purpose of developing microalgal biomass as biofuel. Thus, it is critically important to ensure the extraction process is eco-friendly. Currently, extraction using conventional solvent extraction is more favorable as they are inexpensive and easy to perform. Howbeit, the solvents used such as chloroform are toxic and possess a danger to environment and human. Moreover, the volume of solvent required will become enormous when extraction process is carried out in industrial scale. In this regard, a cleaner and eco-friendly production is vital in present day to avoid exacerbation of environmental pollution. Recently, green solvents such as deep eutectic solvents (105), bio-derived solvent (106), ionic liquids (107) and switchable solvents (108) have been invented. Extraction process of microalgal biomass using recyclable green solvents thereof is recommended to be studied.

Selection of suitable microalgae is a critical factor to achieve the economical mass production. As such, the selected microalgae should be able to produce high amount of desired products while easily to be extracted. Microalgal cell morphology such as thin cell wall, large cell size and filamentous allowed easier separation from the medium. Whereas small cell size and thick cell wall render the harvesting process become costly and energy consuming (109). To select a microalgal strain with high biochemical yield per unit cultivation area, screening and isolating potential microalgae from nature or wastewater can be performed in future study (110; 111). Development of high performance microalgal strains through genetic engineering is another option for making economical feasible microalgae-derived products (112; 113). Several researchers have revealed that genetic engineering can improve the biochemical production of microalgae. For instance, the recombinant strain of Scenedesmus obliquus CPC2-G1 showed successfully increases in biomass and lipid productivity, at 16.3% and 84.9% higher than the wild-type strain (114). In Nannochloropsis salina, overexpressing a bHLH transcription factor led to increase the biomass production with a simultaneous increase of fatty acid methyl esters in lipid (115).

Genetic engineering could also render microalgae to acclimatize the harsh outdoor conditions with desired biochemical production. For example, wild Chlorella sp. is difficult to thrive in the outdoor photobioreactors which

frequently surpasses 40 °C at subtropical or tropical area due to sunlight irradiation during the daytime. After N-methyl-N′-nitro-N-nitrosoguanidine mutagenesis and screening, mutated Chlorella sp. exhibited thermo- and high CO_2-tolerance in the indoor or outdoor photobioreactor with the high biomass and biochemical production (116). However, the genetic modified organisms-derived products are still recognized as negative and not consented by the public opinion. In this regard, more studies including genomics and proteomics analysis are utterly required to exploit the understanding of the underlying genetic engineering and its safety to the environment.

There are various types of available microalgae cultivation system such as open system, closed system, offshore cultivation and dark system. Open and closed systems are the prevalent systems among all the cultivation systems. Open system such as open ponds and raceway ponds offers several benefits such as low operational and capital cost, and minimal energy requirement. Nevertheless, open system is susceptible to high contamination risk, long growth period, low controllable conditions and large area for construction. On the other hand, closed system which mostly referred as bioreactor can overcome the problems of the open system. Closed system provides biomass with better quality as it is performed at controllable conditions. Moreover, the bioreactor can be designed particularly in compliance with the need of microalgae species. On the downside, the closed system requires high cost and high energy to build up the construction and maintain the optimal conditions such as light and temperature. The bioreactor also needs the oxygen management. Too high concentration of oxygen in bioreactor will inhibit the microalgal growth (117). Several literatures have indicated that the closed system can be more efficient when combined with continuous cultures (118; 119). Continuous supply of nutrient can ensure high growth rate of microalgae but not favorable for high lipid production of most microalgae as most microalgae produce lipid during stress. In order to assure high biomass production with desired biochemical, two-stage hybrid system has been suggested (120). In two-stage hybrid system, microalgae are initially cultured in nutrient-rich closed system to increase the cell density. When the microalgae reach the desired cell density, appropriate volume of microalgae culture is transferred into the nutrient-poor open pond to induce biochemical production. Meanwhile, closed-system is replaced with another fresh nutrient medium. The results revealed that two-stage hybrid system is more effective in biomass and biochemical production compared to open and closed systems (120; 121; 122). Moreover, two-stage hybrid system can mitigate the disadvantages of both open and closed systems. In spite of advantages, two-stage hybrid system is more complex and laborious when transferring the microalgae from the nutrient-rich medium to the nutrient-poor medium. In this manner, the development of advanced automate system will reduce the need of manual operation and ensure the uniform transfer of biomass. Moreover, integrating two-stage hybrid system with automation for the auto control of the light intensity, aeration rate and temperature could help to increase the productivity in accordance to the microalgal growth in the closed system. The program such as smart phone application could be developed with automation system to allow operator to change the system when needed.

In most case, one-factor-at-a-time experimental design is still prevailing. Despite of macronutrient, other parameters such as pH (32; 42; 69), temperature (8; 23; 32), metal (123), light intensity (42; 68; 124) , salinity and photoperiod (123) also have critical effects on microalgal growth and biochemical production. With the advancement of information technology, low-cost and effective programming can be developed to allow the performing of factorial design. On the other hand, expensive capital cost is one of the obstacles of microalgal biofuel therefore economic factor should be included in the future to ascertain the best group of combined parameters for the lucrative biomass and biochemical production.

4 Conclusion

Literature studies have confirmed that nitrogen has more pernicious effect than other macronutrients on most microalgal growth and lipid production. Besides, the concentrations and types of macronutrients have remarkable effects on microalgae hence must be chosen scrupulously to achieve desired biomass and metabolite production. High or low supply of nitrogen, carbon or phosphorus have inhibitive effect on microalgal growth but might induce certain metabolite accumulation. There is no universal medium that can be applied to cultivate all the microalgal

strains with high biomass and metabolite production. An efficient medium should be based on microalgal strain and desired metabolite. Normally, microalgae accumulate more lipids under nutrient deprivation, but biomass and other metabolites are also compromised. This situation is not economical as simultaneous production of several metabolites can maximize the profit in concomitant compensate for the cultivation cost. Consequently, an effective strategy should be commenced in the near future to curtail this offset. Despite of concerning on lipid productivity, carbohydrates and proteins need more research and development activities. To obtain high biomass and metabolites accumulation with minimum cost, several innovative methods including wastewater cultivation with flue gas, genetic engineering and automated two-stage hybrid system have been suggested. Additionally, economical factor should be studied in the future using factorial design to confirm the best group of combined parameters for the lucrative biomass and biochemical production.

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